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Research Paper

Hair and Blood Levels of Aluminum, Cadmium, and Lead in Children with Autism from Egypt: Can Toxic Heavy Metals Increase the Risk of Autism?

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ABSTRACT

Background: Autism, a neurodevelopmental disorder that manifests early in childhood but the pathogenic risks are controversial, and some environmental factors are thought to be involved. The association between toxic heavy metals and autism is currently a subject of research, and studies are underway on the role of toxic heavy metals in Egypt, focusing on the social, cultural, and environmental aspects. We investigated the aluminum, cadmium and lead levels in the hair and blood samples of Egyptian autistic children.

Methods: This study was conducted between July 2021 and December 2022 on 32 children with diagnosed autism, aged three to 13 years old, whom were compared with 30 age- and gender-matched children (normal controls). These children were subjected to childhood autism rating scale (CARS), and IQ tests. Also, the aluminum, cadmium, and lead levels were measured in their hair and blood samples for further statistical analyses.

Results: The autistic children had significantly higher levels of aluminum, lead, and cadmium in the hair samples compared to those of the controls. Also, the blood levels of aluminum and cadmium were significantly higher in the autistic children. Those with severe autism had a higher level of hair aluminum compared to those with mild autism. We found positive correlations among the CARS data versus hair aluminum and blood cadmium levels. The regression analyses on blood cadmium levels were also predictive of CARS.

Conclusion: The study findings suggest a likely role for the three heavy metals as being the potential environmental triggers of autism in children.

Keywords: Aluminum; Autism; Cadmium; Egypt; Lead

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by difficulties with social interaction and communication, speech disorder, and the presence of repetitive behaviors [1]. The global incidence of ASD appears to have increased over time, with variations reported in many countries. In 2002, it was estimated that one in 160 children in the United States had ASD, which increased to one in 60 children by 2014. Based on studies conducted in Arab countries, the prevalence of childhood ASD was 11% in Tunisia and 33% in Egypt [2].

Although the etiology of ASD is still uncertain, researchers attribute it to genetic and environmental factors. Heavy metals are considered developmental and reproductive toxins. Exposure to heavy metals, even at low doses, may cause brain impairment. Genetically, autistic children may have reduced ability to detoxify toxic environmental agents [3]. Children tend to be vulnerable to the toxicity of heavy metals for a number of reasons: a) their developing brain is prone to be poisoned with chemical agents; b) their bodies have higher absorption rates and slower detoxification abilities than that of adults; c) the metabolic pathways in fetuses and infants are immature, and, d) their blood-brain barrier is not yet fully developed [4]. Mothers who are chronically exposed to toxic heavy metals could pass them to their fetuses or infants via their placenta or by nursing [5]. The fetal levels of toxic heavy metals have been found to be higher than the actual levels in the maternal blood [6].

Possible sources of toxic heavy metals include drinking water, chemical products, foods such as vegetables and fish, industrial paints, fertilizers, building materials, and cosmetics. Lead is commonly found in paints and can be found in contaminants near roads. Children with pica habits who eat dust or paint chips may develop toxic lead levels in their blood [7]. The association between ASD and heavy metals warrants further research to clarify the controversy over the pathogenesis, as exposure is preventable by treatment and environmental modification.

Aim of the Study: The aim of the present study was primarily to determine the differences in the levels of toxic heavy metals: lead, aluminum, and cadmium in the hair and blood samples of autistic children versus those of the controls. Also, we wondered whether or not these levels could be linked to the severity of ASD.

Materials and Methods

Study Design: The current study was a hospital-based case-control study performed at Fayoum University Hospital's pediatric neurology unit from July 2021 to December 2022. According to the commitment to standard operating guidelines, ethical approval was obtained from the Medical Research Ethics Committee of the Faculty of Medicine, Fayoum University. A consent form was reviewed and signed by one parent of each child, consistent with the institutionally mandated guidelines for experimental research on human subjects.

Sample Size: The study's sample size was determined using, G Power software, version 3.0.10. The minimal sample size was 26 for each group, assuming a power level of 0.80, an alpha level of 0.05 (two-tailed), and a large effect size of 0.8 for the difference between the two study groups on the heavy metal levels. The final sample size consisted of 32 children, ages 3–13, diagnosed with ASD, based on the DSM-5 criteria, and thirty healthy children matched for age and sex as the controls. Children with ASD were recruited from Fayoum University Hospital's pediatric neurology unit. The control children were selected from the outpatient clinic of the pediatric hospital affiliated with Fayoum University, Fayoum, Egypt.

Exclusion Criteria: Patients with any other neurological disorders, a chronic debilitating disease, or whose hair was dyed and bleached were excluded from the study.

Definition of Autism: Autism spectrum disorder (ASD) is defined as deficits in social communication as well as constrictive interests and behaviors. DSM-5 no longer necessitates the presence of deficient language but instead it combines interpersonal deficiencies with communication skills and challenges [8].

Experimental Samples Collection and Analyses

Hair Samples: Hair samples were collected from each of the study children, using clean stainless steel scissors. The hair sample was taken from the back of the head, close to the scalp, in thin slivers from 2-3 locations. The hair samples must have been dry, since wet hair may lead to mould formation. The hair pieces were 4-cm long and weighed approximately one gram each. The hair samples were placed in clean and unused paper envelopes. Then each envelope was labeled and kept closed until performing the analyses.

Hair Sample Preparation: The experimental hair samples were prepared according to Ishak, et al., [9] as follows: All analytical steps were observed in the Agricultural Research Laboratory, Ministry of Agriculture. Using a mechanical shaker, the samples were washed with the following solvents in the next order: 0.5% Triton-X 100, deionized water, and acetone, followed by repetitive rinsing in deionized water. Each solvent was stirred a few minutes. The hair samples were dried using an oven at 60°C (International Atomic Energy Agency, IAEA).

Hair Sample Digestion: Each dried hair sample was placed in a 50-mL beaker and digested in 10 mL of a mixture of 69% concentrated nitric acid and H2O2 (3:1 mL) (Suprapur; Merck, Germany), and left at room temperature overnight. The beaker's contents were then heated on a plate at 100°C to produce a white crystalline residue. It was then re-diluted with deionized water to a final volume of 35 mL.

Hair Sample Analyses: Analysis of the hair samples for heavy metal contents of lead (Pb), aluminum (Al), and cadmium (Cd) was done, using inductively coupled plasma mass spectrometry (ICP-MS/MS: Agilent 8800). The instrument was warmed and tuned up with a diluted ICP-MS tuning solution containing Ce, Co, Li, Tl, and Y (Agilent Technologies). A 200 microgram/L internal standard containing Bi, Ge, In, Li, Lu, Rh, Sc, and Tb (Agilent Technologies) was conducted during the analysis. The instrument was calibrated, using a multi-element standard containing the measuring elements.

Blood Sample Collection: A 5-ml of the venous blood was collected from each of the children, then centrifuged and kept in lead-free tubes at minus 20°C until further analyses. The detection of heavy metals in the blood samples was done using inductively coupled plasma mass spectrometry (ICP-MS) and according to the manufacturer's instructions.

Psychiatric Evaluation: All autistic children were examined for the diagnosis of ASD according to the guidelines of DSM-5 [8]. We performed the childhood autism rating scale (CARS) to quantify the severity of autism in children with ASD. The standard CARS form was used to assess patients younger than six years old with communication difficulties or below-average estimated IQs, while CARS-2-HF (high functioning) form was used for assessing verbally fluent children, six years of age or older, with IQ scores above 80. It consisted of 15 items with a 4-point rating scale, covering a variety of functions. Then the final scores from CARS forms were divided into the following three severity groups:

No ASD symptoms (15-29.5)

Mild-to-moderate ASD symptoms (30-36.5)

Severe symptoms of ASD (37 and higher) [10-12].

The intelligence quotient (IQ) was measured based on the Stanford-Benet Intelligence Scale (5th edition) [13].

Statistical Analyses: The collected data were organized, tabulated, and statistically analyzed using SPSS software, version 22 (SPSS Inc., USA). The Kolmogorov-Smirnov test was performed to determine the normality of the study data. The means and standard deviations were calculated for age, and the unpaired t-test was used to establish the statistical significance of differences among the data groups. Median and interquartile ranges (IQR) were estimated for the levels of heavy metals. The Mann-Whitney or Kruskal-Wallis test was utilized to compare any two or three groups, respectively. Categorical data were presented as numbers and percentages, and the chi-square ($\chi 2$) test was used as a test of significance. Spearman correlation was performed to identify the relationship between heavy metals and the study parameters for blood and hair samples. Two multiple linear regression models were performed for the presence of heavy metals in the hair and blood samples, and as predictors of CARS. For the interpretation of results, the significance level was set at $P\leq 0.05$.

Table 1. Comparison between studied cases and controls regarding characteristics and risk factors.

Age Years (Mean± SD)	**ASD (N=32)**		**Control (N=30)**		**P-value**
	6.7 ± 2.7		**6.4 ± 2.4**		**0.660**
Gender					0.974
Male	18	56.3%	17	56.7%	
Female	14	43.8%	13	43.3%	
Residence					0.987
Rural	17	53.1%	16	53.3%	
Urban	15	46.9%	14	46.7%	
Height					1.000
<5th	5	15.6%	4	13.3%	
5th-95th	27	84.4%	26	86.7%	
Weight					1.000
<5th	2	6.3%	2	6.7%	
5th-95th	30	93.8%	28	93.3%	
Use of Aluminum Pans					0.071
Negative	7	21.9%	13	43.3%	
Positive	25	78.1%	17	56.7%	
Smoking person in home					0.201
Negative	14	43.8%	18	60.0%	
Positive	18	56.3%	12	40.0%	
Source of water					0.455
No lead pipe	23	71.9%	24	80.0%	
Lead pipe	9	28.1%	6	20.0%	
Consanguinity					0.647
Negative	21	65.6%	18	60.0%	
Positive	11	34.4%	12	40.0%	
Similar conditions					0.396
Negative	28	87.5%	29	96.7%	
Positive	4	12.5%	1	3.3%	

Table 2. IQ and CARS in ASD group.

Category	**Median**	**IQR**
IQ test	67	60-82
CARS score	39.5	34-44.5
IQ interpretation	N	%
Mental retardation	20	62.5%
Below average mentality	10	31.3%
Normal mentality	2	6.3%
CARS interpretation		
Mild- Moderate	13	40.6%
Severe	19	59.4%

IQR: Inter-quartile range; Al: Aluminum; Pb: Lead; Cd: Cadmium; IQ: Intelligence quotient

Table 3. Comparison of ASD cases versus controls for the blood and hair levels of Al, Pb and Cd.

	ASD			**Control**			**P-value**
	Median	**IQR**		**Median**	**IQR**		
Hair Alum mg/kg	33.13	23.37	61.3	11.79	10.11	16.65	<0.001*
Hair Lead mg/kg	2.40	2.13	3.27	1.51	1.23	2.14	<0.001*
Hair Cd mg/kg	2.91	2.14	4.05	0.47	0.25	0.98	<0.001*
BL Alum µg/dl	1.37	1.04	2.17	1.05	1.02	1.24	0.007*
BL lead µg/dl	1.03	0.44	1.10	0.75	0.37	1.04	0.131
BL Cd µg/dl	0.77	0.33	1.06	0.31	0.14	0.48	<0.001*

* Highly Significant; AL: aluminum; Pb: lead; Cd: Cadmium; IQR: Inter-quartile range

Table 4. The relation between hair levels of heavy metals in ASD group and patient characteristics.

	Hair Alum µg/dl			Hair Lead µg/dl			Hair Cd µg/dl		
	Median	IQR	P-value	Median	IQR	P-value	Median	IQR	P-value
Residence			0.411			0.628			0.009**
Rural	27.23	23.28	44.34	2.3	1.66	3.3	2.44	1.12	3.04
Urban	44.35	23.45	70.03	2.47	2.2	3.24	4.01	2.86	4.45
Use of aluminum Pan			<0.001**			0.420			0.224
Negative	15.37	11.11	22.32	2.13	1.16	3.3	1.12	1.05	4.04
Positive	44.34	27.12	66.24	2.45	2.2	3.16	3.04	2.44	4.06
Smoking person in home			0.301			0.985			<0.001**
Negative	30.17	22.32	54.3	2.4	2.13	3.3	2.25	1.06	2.56
Positive	34.14	24.71	70.03	2.39	2.12	3.24	4.04	2.96	4.45
Source of water			0.198			0.967			0.170
No lead pipe	35.15	24.71	66.24	2.34	2.13	3.6	3.08	2.44	4.12
Lead pipe	26.3	15.24	55.48	3.05	1.66	3.16	2.25	1.12	3.04
Consanguinity			0.238			0.434			0.725
Negative	37.25	23.45	70.03	3.04	2.12	3.3	3.04	2.24	4.06
Positive	25.3	23.28	54.3	2.3	2.13	3.05	2.65	2.04	4.04
Similar conditions			0.721			0.332			1.000
Negative	33.13	23.37	65.79	2.41	2.13	3.27	2.91	2.245	4.05
Positive	31.07	20.51	47.25	1.75	1.03	3.34	3.04	1.54	4.76
CARS interpretation			0.018*			0.545			0.448
Mild	23.45	22.14	37.25	3.05	2.13	3.3	2.56	2.04	4.03
Severe	44.34	26.3	70.25	2.3	1.44	3.15	3.04	2.25	4.12
IQ interpretation			0.972			0.109			0.465
Mental retardation	33.13	24.8	61.3	2.3	1.55	3.09	2.76	2.145	4.04
Below average mentality	30.35	22.14	77.32	3.27	2.24	4.6	3.73	2.04	5.03
Normal mentality	35.79	27.23	44.35	2.24	2.13	2.34	2.40	2.24	2.56

Significant; ** highly significant; IQR: Inter-quartile range; AL: aluminum; Pb: lead; Cd: Cadmium; IQ: Intelligence quotient.

Table 5. The relation between blood levels of heavy metals in ASD group and patient characteristics.

	Blood Alum mg/kg			Blood Lead mg/kg			Blood Cd mg/kg		
	Median	IQR	P-value	Median	IQR	P-value	Median	IQR	P-value
Residence			0.433			0.264			0.020*
Rural	1.24	1.22	2.06	0.54	0.45	1.04	0.45	0.32	0.77
Urban	2.05	0.58	3.05	1.04	0.42	1.30	1.04	0.45	1.34
Use of aluminum Pan			<0.001**			0.474			0.656
Negative	0.54	0.45	1.01	0.35	0.33	1.64	0.46	0.22	1.06
Positive	2.05	1.23	3.05	1.03	0.45	1.05	0.77	0.34	1.06
Smoking person in home			0.694			0.613			0.001**
Negative	1.43	1.01	2.13	0.85	0.45	1.05	0.41	0.24	0.54
Positive	1.37	1.04	3.05	1.03	0.42	1.3	1.05	0.77	1.34
Source of water			0.157			0.536			0.409
No lead pipe	1.43	1.23	3.05	1.03	0.45	1.12	0.87	0.32	1.07
Lead pipe	1.03	0.54	2.06	1.03	0.35	1.05	0.76	0.45	1.02
Consanguinity			0.506			0.208			0.845
Negative	1.43	1.22	3.05	1.04	0.45	1.12	0.76	0.32	1.07
Positive	1.30	1.01	2.10	0.60	0.35	1.05	0.77	0.34	1.04
Similar conditions			0.254			0.392			0.361
Negative	1.82	1.04	2.63	1.03	0.44	1.12	0.77	0.32	1.05
Positive	1.23	0.89	1.27	0.57	0.43	0.82	0.80	0.45	1.63
CARS interpretation			0.195			0.880			0.126
Mild	1.25	1.01	2.05	1.04	0.35	1.05	0.46	0.24	1.02
Severe	2.03	1.04	3.12	0.66	0.45	1.30	1.03	0.34	1.07
Mental retardation	1.45	1.04	2.17	0.63	0.43	1.04	0.77	0.34	1.04
IQ interpretation			0.843			0.312			0.070
Below average mentality	1.34	1.01	3.60	1.08	0.35	2.04	1.05	0.46	1.34
Normal mentality	1.70	1.25	2.14	0.75	0.45	1.04	0.19	0.13	0.24

Table 6. Correlation between IQ and CARS with heavy metals levels in hair and blood in ASD cases and control.

	IQ Test	CARS Score
Hair Alum mg/kg		
r	-0.206	0.447
P-value	0.258	0.010*
Hair Lead mg/kg		
r	0.135	-0.062
P-value	0.463	0.737
Hair Cd mg/kg		
r	-0.060	0.122
P-value	0.742	0.507
BL Alum µg/dl		
r	-0.022	0.220
P-value	0.905	0.227
BL lead µg/dl		
r	0.056	0.098
P-value	0.760	0.593
BL Cd µg/dl		
r	-0.202	0.355
P-value	0.268	0.046*

*Significant; AL: aluminum; Pb: lead; Cd: Cadmium; IQ; Intelligence quotient

Table 7. Multiple Linear regression models for hair and blood heavy metals levels as predictors for CARS

	B	t	P-value	95.0% CI for B	
Model 1					
(Constant)	36.723	12.73	<0.001	30.814	42.632
Hair Alum mg/kg	0.093	1.955	0.061	-0.004	0.19
Hair Lead mg/kg	-0.671	-1.179	0.248	-1.838	0.495
Hair Cd mg/kg	0.444	0.508	0.616	-1.349	2.238
Model 2					
(Constant)	35.786	15.292	<0.001	30.992	40.579
BL Alum µg/dl	1.403	1.459	0.156	-0.567	3.374
BL lead µg/dl	-2.841	-1.165	0.254	-7.838	2.155
BL Cd µg/dl	5.441	2.233	0.034*	0.45	10.432

*Significant; Model 1: CARS was the dependent variable, hair Alum, lead. & Cd were the independent variables. R^2=0.149, F=1.632, p=0.204. Model 2: CARS was the dependent variable, blood Alum, lead.
& Cd were the independent variables. R^2=0.243 F=3.004, p=0.047.

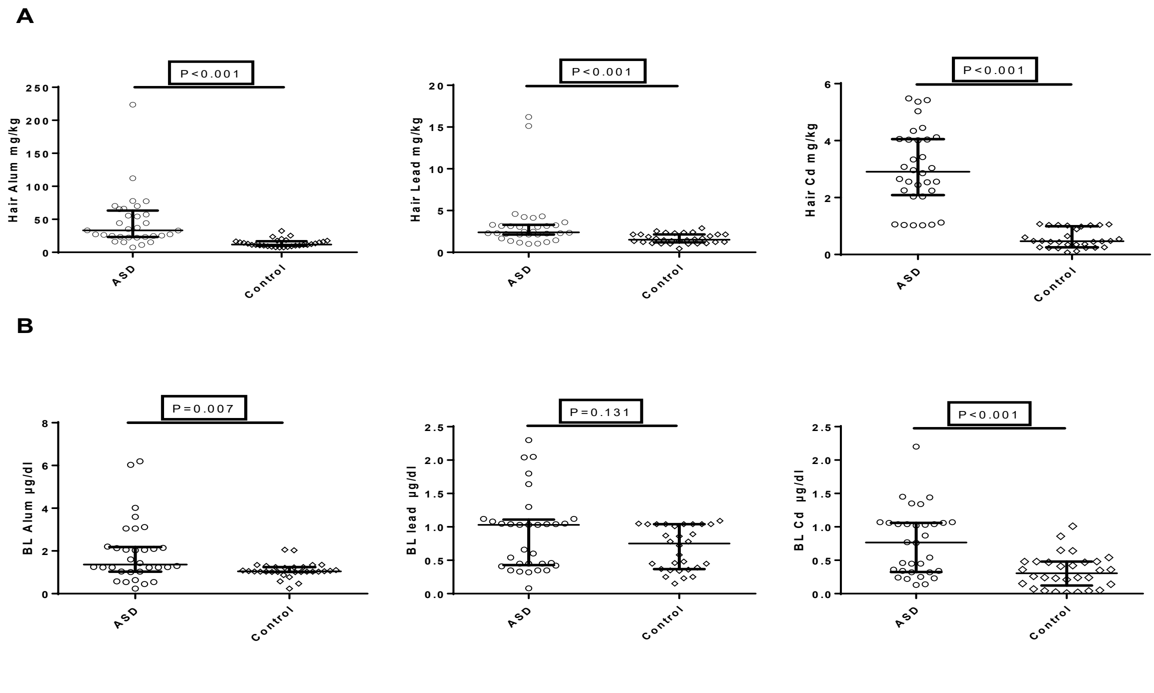

Figure 1. Dot plot with median and IQR of aluminum, lead and cadmium levels in both study groups; A: hair; B: blood.

Results

This study recruited 62 children: 32 with ASD and 30 healthy controls. There were no statistically significant differences in age and gender between the two groups. The use of aluminum cooking pans and the presence of smokers in the ASD group were greater than those of the control group, but the differences were not statistically significant. Similarly, there were no statistically significant differences in other demographic characteristics between the ASD cases and the controls (Table 1).

Among children with ASD, the median for IQ and CARS scores were 67 (60-82) and 39.5 (34-44.5), respectively. Most children with ASD (62.5%) had mental retardation, while 31.3% had below-average mentality, and 6.3% had normal mental status. In terms of interpreting the CARS data, the majority of ASD children (59.4%) were classified as having severe autistic disorders, while 40.6% were classified as having a mild rating for autism (Table 2).There were significantly higher levels of aluminum, lead, and cadmium in the hair samples of ASD children as compared to the controls. On the other hand, the levels of Al and Cd in the blood samples were significantly higher in ASD children compared to those of the controls; however, the difference was not statistically significant (Table 3 and Figure 1).

Among the ASD group, inhabitants of urban areas had significantly higher levels of hair cadmium compared to those of rural areas. Similarly, children who lived with a smoking family member had significantly higher levels of hair cadmium than those living with a non-smoking family member. Children who lived in families that used aluminum pans and pots for cooking had significantly higher levels of aluminum in their hair than those who did not. Children with severe autism had a higher median level of aluminum in the hair compared to those with mild autism (Table 4).

Among children with ASD, residents from the urban areas had significantly higher levels of blood cadmium as compared to those from rural areas. Also, a highly significant difference was documented in the median level of blood cadmium in children who were living with a smoking family member compared to those living with a non-smoking one. Children living in families that used aluminum pots and pans for cooking had a highly significant difference in the level of blood aluminum compared to those who did not use them (Table 5). The aluminum contents in the hair samples were positively correlated with the CARS scores ($r = 0.447$, $p = 0.010$). Likewise, there was a positive correlation between the blood cadmium and the CARS score ($r = 0.35$, $p = 0.046$) (Table 6).A multiple linear regression for the heavy metal levels in the hair samples (model 1) indicated that the model could not predict the CARS status significantly ($R2 = 0.149$, $p = 0.204$). However, the regression model for blood cadmium (model 2) was significantly predictive of the CARS scores ($R2 = 0.243$, $p = 0.047$). Therefore, for every unit of increase in the blood cadmium level, there was an increase in the CARS score of 5.441 (95 % CI: 0.45–10.432) (Table 7).

Discussion

The rise in the prevalence of ASD cannot be entirely attributed to diagnostic advancements or abrupt genetic changes. Scientists and clinicians are increasingly in agreement that ASD results from the interactions between biological susceptibility of individuals and environmental factors [14]. Previous studies have reported the link between certain heavy metals and the etiology of ASD in Egyptian children [5, 7]. To our knowledge, this is the first study to report the levels of the three heavy metals (Al, Cd &Pb) in the hair and blood samples of children with ASD in Fayoum province, Egypt. This geographic area has the typical location, climate, different sources of water supply, and economic status that may promote ASD. The present study was conducted on 62 children, of whom 32 were autistic with a mean age of 6.7 years. Similar findings have been reported by an earlier study [5].

In the current study, male children made up 56.2% of the participants, while females made up 43.8%, with a male-to-female ratio of 1.3:1. Other studies [6, 15, 16] have reported higher male ratios of 3:4, 15:1, and 2.1:1, respectively. The corresponding international ratio is 4:1, which could be attributed to biases preventing female diagnosis and the fact that the true ratio is lower [17].

Results from the present study showed significantly higher levels of the heavy metals (Al, Cd & Pb) in the hair samples of autistic children compared to those of the healthy controls. Also, significantly higher blood levels of Al and Cd were found in autistic children. These subjects had higher levels of blood lead than that of the controls, but it was not statistically significant. Heavy metals including Al, Cd and Pb, tend to concentrate in the air and the food chain along with other toxic metals, resulting in poisoning, which is the commonest environmental hazard [6].

This study provides experimental support for the hypothesis that heavy metals contribute to the etiology of autism. Also, studies have previously reported that heavy metals, such as Al, Pb, As, Cd, and Hg, are associated with ASD [18-20], while few studies have found no such associations [21, 22]. The most likely explanation for the associated toxicities is likely to be exposure to heavy metals through drinking water, food chains, and air pollutants, such as combustive emissions, car exhausts, soil, fertilizers, and paint chips. However, autistic children are not the only ones exposed to these toxic environmental hazards; normal children are also exposed. The likely explanation is that autistic children may lack the ability to detoxify environmental toxins, resulting in the accumulation of toxic metals in their bodies. This phenomenon is known as genetic-environmental interaction.

Moreover, the mean blood lead level in the current study was not significantly higher in the autistic children compared to the controls. Rather, it could be attributed to environmental pollution by lead. Lead exposure is primarily from drinking water, paint chips, soil, fertilizers, food, ceramics, cosmetics, gasoline, newsprint, rubber toys, tap water, tobacco smoke, and dust [6].

Researchers have found that heavy metals, such as mercury, cadmium, lead, and aluminum, affect synaptic transmission in the brain and the central and peripheral nervous systems. Those metals are known to disrupt the calcium level in the brain cells that markedly affect such brain functions as calcium-dependent neurotransmitter release. The disruption results in a depressed level of acetylcholine, serotonin, and norepinephrine, which affects the person's mood and motivation [23]. Exposure to certain toxic substances during developmental periods may contribute to the etiology of autism and may exacerbate the related symptoms [3]. The findings of this study are consistent with the latter fact, where hair Al significantly correlated with CARS scores (severity of autism). Moreover, a positive correlation existed between blood Cd levels and CARS scores.

Adams, et al. [24] also found a significant correlation between the body burden of toxic heavy metals and the severity of autism. Further, that study concluded that the excretion pathways of toxic metals may differ significantly between study subjects with moderate to severe autism and those with mild ASD. Neither the current study nor Elsheshtawy, et al. [7] found a significant correlation between lead exposure and CARS scores. In addition, none of the three tested heavy metals had a significant correlation with IQ. In a previous study [25], it was confirmed that elevated lead levels in the blood resulted in low IQ. The latter findings, however, contradicted this conclusion. The current study found that the ASD group used more aluminum cooking pans than did the control group, but the difference in the scores between the two groups was insignificant. Children from families that used these pans had higher aluminum levels in their hair and blood samples compared to those who did not use them. Similarly, Mohamed, et al. [5] reported the same results.

Experimentally, Abu-Taweel, et al. [26] found that prenatal exposure to aluminum including the use of aluminum during pregnancy and lactation, may affect the development of in utero fetus of mice, indicating that aluminum exposure poses potential and long-term neurotoxic risks. Aluminum induces oxidative stress in the brain tissue, thereby aggravating the clinical manifestations of autism through microglial priming and worsening excitotoxicity [27].Common sources of aluminum exposure include cookware, processed foods and cheese, medications (anti-diarrheal agents), tap water, and softened water. Clinically, high aluminum levels in the blood are known to be linked to encephalopathy [6].

Cadmium is a toxic heavy metal, the intoxication of which is usually due to cigarette smoke, food, water, air contamination, and inappropriately discarded batteries. Urban areas with unregulated industries and construction activities are facing the problem of toxic dust and the accompanying heavy metals. High levels of cadmium from combustive emissions, incinerators and car exhausts are also accumulating in urban areas [28]. The findings of the current study are consistent with the latter facts, where among the ASD group; inhabitants from urban areas had higher levels of cadmium in the hair and blood samples than their rural counterparts. Similarly, autistic children living with smoking parents had higher levels of cadmium in the hair and blood samples than those living with nonsmoking parents.

Conclusions

The findings of the current study established that significantly higher levels of toxic heavy metals in the hair (Al, Cd & Pb) and significantly higher blood levels of Al and Cd were observed in autistic children compared to those of the control group. Hair aluminum and blood cadmium levels were found to be positively correlated with the severity of ASD. Higher levels of the heavy metals in autistic children suggest their role as environmental risk factors responsible for the occurrence of autism. Therefore, chelation therapy and strong public health education against heavy metals should be considered in the management of children diagnosed with ASD.

Conflicts of Interest

The authors declare that they have no conflict of interests.

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Ethical Considerations

All procedures performed in the study were in line with ethical standards of the Faculty of Medicine, Fayoum University. The ethical committee approved the research protocol, under the code R389/ 101/12/2022. Informed consent to participate in this research was obtained from all participating parent or family before being included in the study.

Authors' Contributions

Dr. Amro Saleh: corresponding author, conceptualization of the research idea, methodology, Proofreading and revision of the whole research in addition to plagiarism check, and references reformatting. Dr. Huda El-Kady: collection of hair and blood samples, collection of other included data, ethical committee approval, and reviewing the manuscript. Dr. Mohamed Masoud: Methodology, statistical analysis of the data, and writing the drafts of the manuscript's Tables. Dr. Eman Abdelfatah: follow up with the lab specialists till receiving the results, writing the abstract, Introduction, and discussion.

All authors read and approved the final manuscript.

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